

## Late Larval Development and Onset of Symbiosis in the Scleractinian Coral *Fungia scutaria*

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**Abstract.** Many corals that harbor symbiotic algae (zooxanthellae) produce offspring that initially lack zooxanthellae. This study examined late larval development and the acquisition of zooxanthellae in the scleractinian coral *Fungia scutaria*, which produces planula larvae that lack zooxanthellae. Larvae reared under laboratory conditions developed the ability to feed 3 days after fertilization; feeding behavior was stimulated by homogenized *Artemia*. Larvae began to settle and metamorphose 5 days after fertilization. In laboratory experiments, larvae acquired experimentally added zooxanthellae by ingesting them while feeding. Zooxanthellae entered the gastric cavity and were phagocytosed by endodermal cells. As early as 1 h after feeding, zooxanthellae were observed in both endodermal and ectodermal cells. Larvae were able to form an association with three genetically distinct strains of zooxanthellae. Both zooxanthellate and azooxanthellate larvae underwent metamorphosis, and azooxanthellate polyps were able to acquire zooxanthellae from the environment. Preliminary evidence suggests that the onset of symbiosis may influence larval development; in one study symbiotic larvae settled earlier than aposymbiotic larvae. Protein profiles of eggs and larvae throughout development revealed a putative yolk protein doublet that was abundant in eggs and 1-day-old larvae and was absent by day 6. This study is the first to examine the onset of symbiosis between a motile cnidarian host and its algal symbiont.

### Introduction

The life history of symbiotic associations between organisms necessarily includes a stage during which a new gen-

eration of hosts first acquires its symbionts (Douglas, 1994). Symbionts may be acquired either vertically, whereby the symbiont is transmitted directly from parent to offspring, or horizontally, whereby the offspring must acquire symbionts from the environment (Trench, 1987). Vertical transmission ensures that offspring are provided with a complement of symbionts, whereas horizontal transmission is more uncertain; environmental variability may prevent contact between symbiont and host, resulting in the failure of the host to become infected by its symbiont.

Many members of the phylum Cnidaria (such as corals, sea anemones, and jellyfish) harbor intracellular photosynthetic dinoflagellates (*Symbiodinium* spp.) in a mutually beneficial symbiotic association. The dinoflagellates, also known as zooxanthellae, contribute to host nutrition by translocating photosynthetically fixed carbon, while the hosts provide the zooxanthellae with nutrients and a protected, high-light environment. Many cnidarian host species are obligately symbiotic with zooxanthellae, thus vertical transmission might be predicted to be the dominant mode of symbiont transmission. However, this is not the case, at least in scleractinian corals. Most scleractinian coral species spawn gametes that are azooxanthellate (*i.e.*, lack zooxanthellae) (Fadlallah, 1983; Babcock and Heyward, 1986; Harrison and Wallace, 1990; Richmond and Hunter, 1990; Richmond, 1997). The gametes are fertilized within the water column and develop into azooxanthellate planula larvae that must acquire zooxanthellae at some stage of their development (Trench, 1987).

Offsetting the uncertainty of infection *via* horizontal transmission is the benefit that acquisition of symbionts from the environment might allow the host to form an association with genetically distinct symbionts that are adapted to local conditions. Rowan and Knowlton (1995) found that the corals *Montastraea faveolata* and *M. annu-*

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*laris* naturally associate with several species of *Symbiodinium* that occur along an environmental gradient, and that hosts can contain two species at one time. Many studies have compared the uptake and influence of heterologous and homologous zooxanthellae on experimentally infected cnidarian host polyps (literature summarized most recently in Davy *et al.*, 1997). Thus although vertical transmission ensures that offspring are provided with zooxanthellae, horizontal transmission may allow for the acquisition of symbionts that are adapted to the specific environment in which the offspring ultimately settle.

There are several mechanisms by which initially azooxanthellate cnidarian hosts may acquire their algal symbionts from the environment and incorporate them into endodermal cells, where they ultimately reside. First, zooxanthellae may be incorporated into the embryo. Second, zooxanthellae may be incorporated into the host's ectoderm and then migrate into the endoderm. Both these mechanisms occur in the scyphozoan *Linuche unguiculata*; both embryos and young, nonfeeding planulae are capable of becoming infected by experimentally added zooxanthellae (Montgomery and Kremer, 1995). Third, zooxanthellae may be incorporated directly into endodermal cells, as was first described in scyphistomae (post-planula polyps) of *Cassiopeia xamachana* (Trench, 1980; Fitt and Trench, 1983a, b). In this third mode of infection, symbionts enter through the mouth of the host and are phagocytosed by endodermal cells lining the gastric cavity (Colley and Trench, 1983; Fitt and Trench, 1983a, b).

Although many studies have either documented in detail or anecdotally noted zooxanthella acquisition by naturally azooxanthellate polyps (scyphozoans; Sugiura, 1964; Trench, 1980; Colley and Trench, 1983; Fitt and Trench, 1983a, b; anthozoans; Kinzie, 1974; Babcock and Heyward, 1986; Benayahu *et al.*, 1989), little information exists about either the life-history events or the mechanisms of infection associated with the onset of symbiosis in initially azooxanthellate planulae. Montgomery and Kremer (1995) found that young planulae of the scyphozoan *Linuche unguiculata* became infected (by an unknown mechanism) by experimentally added zooxanthellae. Schwarz (1996) found that planulae of the temperate sea anemone *Anthopleura elegantissima* acquired zooxanthellae *via* phagocytosis after feeding on animal tissue that contained zooxanthellae recently isolated from a previous host.

Given that most scleractinian corals produce azooxanthellate planulae, it is likely that at least some acquire zooxanthellae during the planula stage. Planulae are motile and represent the dispersal stage of corals; the acquisition of symbionts during this stage might therefore be advantageous because it presents an opportunity for the planulae to acquire symbionts adapted to the environment in which the larval hosts will settle live.

In this study we examined the process of symbiont ac-

quisition in the scleractinian coral *Fungia scutaria*. This solitary coral is gonochoric, and the females spawn azooxanthellate eggs that are fertilized within the water column and develop into azooxanthellate larvae (Krupp, 1983). Krupp reported on the early development of this species and observed that larvae reared in aquaria with adult corals acquired zooxanthellae 4 to 5 days after spawning. He observed a "mouth opening" response to the addition of zooxanthellae obtained from homogenized tissues of adult *F. scutaria*, but did not observe zooxanthellae entering the mouths of the larvae. In this paper we describe late larval development and the process of symbiont acquisition (infection) in *F. scutaria*, including the developmental stages at which the host is competent to become infected by zooxanthellae, the mechanisms by which the zooxanthella are acquired and incorporated into host tissue, the effect of feeding behavior on the infection rate, the specificity of the host-symbiont relationship, and the protein profiles of azooxanthellate and zooxanthellate larvae through development.

## Materials and Methods

### *Gamete collection and larval cultures*

About 75 adult specimens of *Fungia scutaria* are maintained year-round in running seawater tables at the Hawaii Institute of Marine Biology on Coconut Island, Kaneohe Bay, Hawaii. For our experiments, the corals were rinsed with seawater and placed in standing seawater in individual glass finger bowls. Filtered (0.45  $\mu\text{m}$ ) seawater was used for all cultures. This species generally spawns between 1700 and 1900 h, 2–4 days after the full moon during June through August. In August 1995, August 1996, and June and July 1997, eggs were collected by removing the adults from the finger bowls and leaving the eggs in the bowl into which they were spawned. If the egg density was greater than a single layer of eggs at the bottom of the dish, some of the eggs were collected with a turkey baster and transferred to a new finger bowl. Within 30 min after spawning, water from the dishes of all spawning males was combined and a small volume was gently pipetted into the dishes containing eggs. The dishes were left in a seawater table overnight for fertilization and early larval development. The following day, the water was changed. Larvae from all parental crosses were combined, and the larvae were maintained in large glass finger bowls in filtered seawater, which was changed every day.

### *Preparation of zooxanthella isolates*

Zooxanthellae were isolated from adult specimens of *F. scutaria* by using the spray from an oral hygiene device (Water Pik) to remove and homogenize coral tissue; they were then concentrated using a tabletop centrifuge at

2000 × g. The zooxanthella pellet was rinsed twice in filtered seawater to partially clean it of animal tissue and was again concentrated by centrifugation. Zooxanthella isolates were used within 2 h of preparation. The same methods were used to isolate zooxanthellae from the sea anemone *Aiptasia pallida*, except that whole animals were homogenized in a ground-glass tissue grinder.

#### Preparation of homogenized *Artemia* sp.

To stimulate feeding behavior in larvae, homogenized *Artemia* sp. (brine shrimp) was added to larval cultures. A small pinch of frozen *Artemia* was homogenized in a ground-glass tissue grinder in about 1 ml of seawater and filtered through a 60- $\mu$ m mesh to remove large particulate matter. The resulting slurry was used within 15 min of preparation.

#### Acquisition of zooxanthellae

To identify (a) the developmental stages at which *F. scutaria* is competent to become infected and (b) the mechanisms of zooxanthella acquisition, larvae from four stages of development (Table 1) were exposed to zooxanthellae from different sources, with or without homogenized *Artemia* (a feeding stimulant). Homologous algae were freshly isolated from adult *F. scutaria*, and heterologous algae were either freshly isolated from the sea anemone *Aiptasia pallida* or taken from algal cultures originating from the jellyfish *Cassiopeia xamachana*. Three replicates were established for all treatments. Larvae were concentrated in glass finger bowls (>10<sup>3</sup> larvae per bowl), and an even layer of zooxanthellae was pipetted along the bottom of the bowls. Several drops of homogenized *Artemia* were added to the appropriate treatments. Zooxanthellae and *Artemia* slurry were removed either 4 or 24 h later (Table 1) by concentrating larvae on a filter and placing them into clean filtered seawater. Some larvae from each treatment were observed under a compound microscope, either immediately after

zooxanthellae were removed or 24 h later, to determine if they had become infected with zooxanthellae.

For treatments C1 and C2 (Table 1), we used the following method to determine the fraction of larvae that became infected. Twenty-four h after the larvae were exposed to zooxanthellae, the water in the larval cultures was swirled and one aliquot was removed from each replicate. Between 25 and 56 larvae per aliquot were examined under a compound microscope to count how many contained zooxanthellae.

#### Larval development

To observe and quantify the developmental progression of both azooxanthellate and zooxanthellate larvae, six replicate cultures of each were maintained in plastic 6-well culture dishes (300–500 larvae per well in 5 ml of filtered seawater). Water was changed roughly once a day. Larval development was monitored for about 2 weeks. Each replicate well was placed haphazardly under a dissecting microscope, and within the field of view, the number of larvae at each developmental stage was counted.

#### Electron microscopy

To follow the process of zooxanthella incorporation into host tissue, larvae from treatment C2 were sampled and fixed for electron microscopy 1 and 24 h after zooxanthellae were added to larval cultures. The larvae were placed in sampling cups, which were prepared by cutting off the bottoms of microfuge tubes and affixing 50- $\mu$ m mesh across the bottom. The cups were placed in 1% glutaraldehyde in phosphate-buffered saline (PBS, 0.1 M sodium phosphate, 0.45 M sodium chloride, pH 7.2) for 1 h; rinsed 3 × 10 min in PBS; postfixed for 1 h in 1% osmium tetroxide in PBS; rinsed 3 × 10 min in PBS; and dehydrated for 15 min each in 30%, 50%, and 70% ethanol, and then 1 h each in 80%, 95%, and 3 × 100% ethanol. Samples for scanning electron microscopy were dried for 15 min in hexamethyldisilane.

Table 1

#### Experimental treatments of *Fungia scutaria* larvae

Treatment: Developmental stage	Source of Algae	<i>Artemia</i> added?	Exposure duration	Infection determined
A: embryo-early planula (0–12 h old)	<i>F. scutaria</i>	no	overnight	immediately
B: early planula (1–2 days old)	<i>F. scutaria</i>	no	overnight	immediately
C1: fully developed planula (3 days old)*	<i>F. scutaria</i>	no	4 h	after 24 h
C2: fully developed planula (3 days old)*	<i>F. scutaria</i>	yes	4 h	after 24 h
D1: fully developed planula (3 days old)*	<i>A. pallida</i>	no	4 h	after 24 h
D2: fully developed planula (3 days old)*	<i>A. pallida</i>	yes	4 h	after 24 h
E1: fully developed planula (3 days old)*	<i>C. xamachana</i>	no	4 h	after 24 h
E2: fully developed planula (3 days old)*	<i>C. xamachana</i>	yes	4 h	after 24 h
F: polyp (after metamorphosis)	<i>F. scutaria</i>	no	overnight	after 24 h

\* Planulae were considered fully developed once they had acquired the ability to feed.

mounted on stubs, coated with 60:40 Au:Pd, and viewed on an Amray 3300FE scanning electron microscope. Samples for transmission electron microscopy were infiltrated with Spurr's resin in 1:1 ethanol:resin for 2.5 h, 1:3 mix for 2.5 h,  $2 \times 100\%$  resin for 1 h, and 100% resin overnight at  $60^\circ\text{C}$ . Thin sections were prepared on an ultramicrotome, stained with uranyl acetate and lead citrate, and viewed on a Philips CM12 transmission electron microscope.

#### Polyacrylamide gel electrophoresis

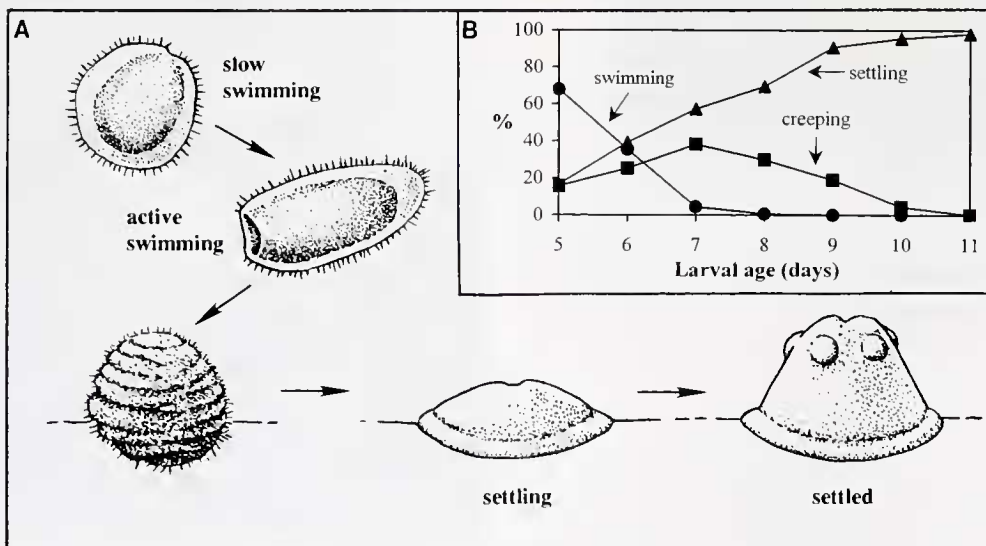
We prepared one-dimensional SDS-PAGE protein profiles of both azooxanthellate and zooxanthellate larvae through development (eggs through 6-day-old larvae). For each sample, about 1000 larvae were counted, collected by centrifugation, and frozen at  $-80^\circ\text{C}$ . Protein extracts were prepared by homogenizing frozen larvae over ice in a ground-glass grinder in 100  $\mu\text{l}$  of homogenization buffer (40 mM Tris-HCl, 10 mM EDTA, protease inhibitor cocktail (Sigma), pH 7.4). Homogenates were centrifuged for 10 min at  $14,000 \times g$  to pellet zooxanthellae and animal debris. The protein concentration of the supernatant was determined spectrophotometrically (Bradford, 1976); larvae contained approximately 50–100 ng protein/larva. Larval proteins were resolved on 12.5% SDS-PAGE gels under reducing conditions (methods modified from Laemmli, 1970). Gels were silver stained (methods modified from Heukeshoven and Dernick, 1986) and scanned on an Imagemaster desktop scanner (Pharmacia) and analyzed using Imagemaster software (Pharmacia).

## Results

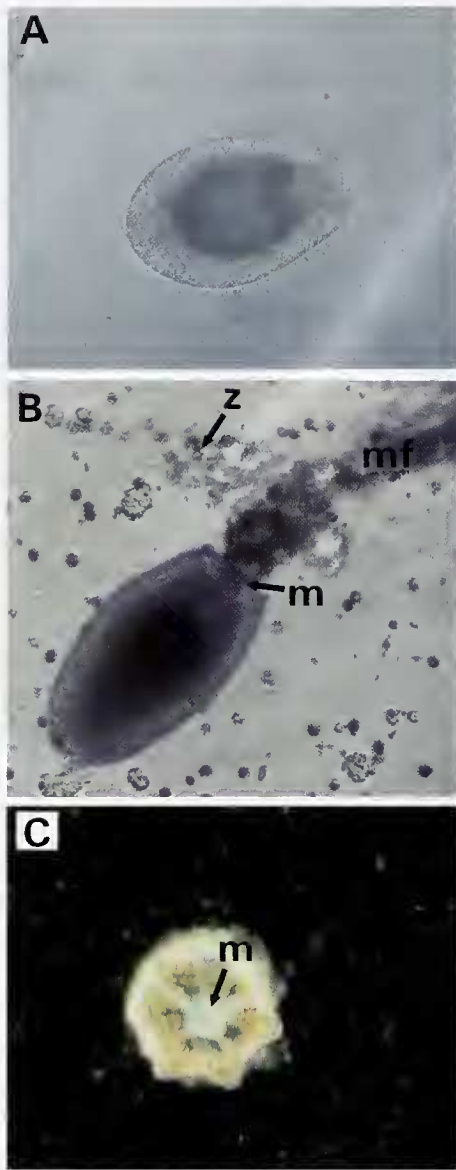
### Larval development

Larval development was observed over three summers (1994, 1996, 1997). Larvae from all years followed the same progression of developmental stages, as illustrated in Figure 1A and detailed below in Figure 2, progressing from swimming to creeping to settled. The duration of each developmental stage, however, was variable; for the later stages it differed by up to several days both within and among replicates. Figure 1B shows the time course of developmental events for zooxanthellate larvae from 1996.

All larvae progressed through the following series of stages. Within 12 h after fertilization, slowly moving, ciliated spherical planulae developed; within 24 h, barrel-shaped planulae, roughly 100  $\mu\text{m}$  in length (shown in Fig. 2A), had developed and were actively swimming at all depths in the culture dishes. By day 3, larvae had fully formed mouths and functional gastric cavities, and were capable of feeding. Upon addition of food (homogenized *Artemia*), larvae ceased swimming and dropped to the bottom of the dish. They extruded mucus, their oral ends expanded, and they ingested whatever they landed on, including experimentally added zooxanthellae. As they fed, their gastric cavities became filled with particulate matter (Fig. 2B). Some larvae resumed swimming while trailing a strand of mucus; the mucus trapped particulate matter that slowly entered the mouth. Larvae continued to feed for several hours and then resumed swimming. Except for



**Figure 1.** Progression of developmental events in *Fungia scutaria* larvae. (A) Schematic representation of developmental stages from the early planula through metamorphosed polyp. (B) Example of the time course of developmental events. Data shown are from zooxanthellate larvae in 1996. Larvae were infected with zooxanthellae on day 3 and then divided into six replicate dishes, which were monitored daily. Each point represents data pooled from the six replicates. Larvae progressed from swimming to creeping to settling.

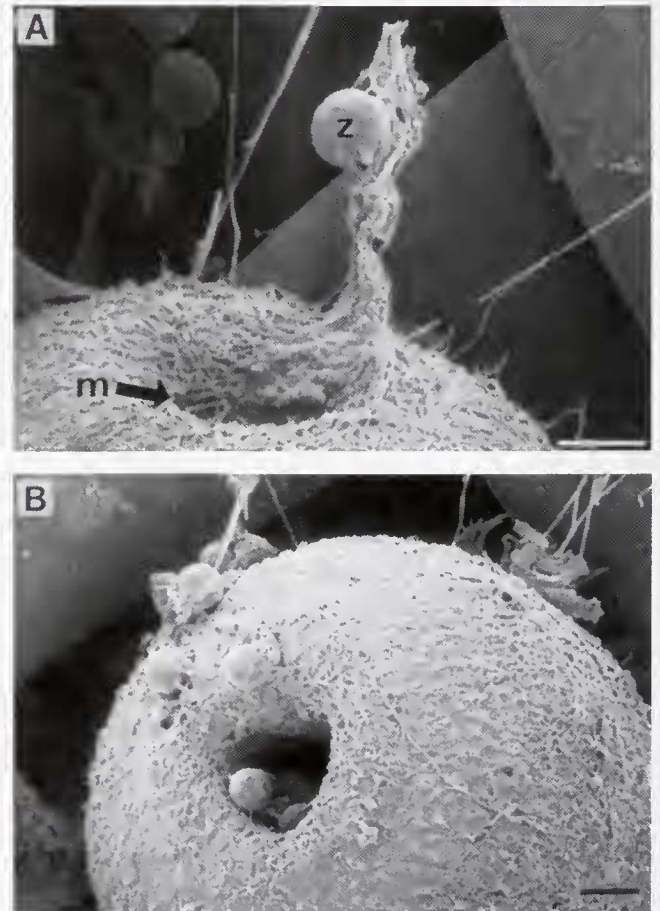


**Figure 2.** Light micrographs of stages in the development of *Fungia scutaria* larvae. (A) Two-day-old planula larva, prior to development of a mouth. (B) Three-day-old feeding planula (m = mouth, mf = mucous strand with food particles attached, z = zooxanthella). (C) Polyp with tentacles, 6 days after settling. Zooxanthellae are visible as golden spheres. Planula length and polyp diameter, approximately 100  $\mu\text{m}$ .

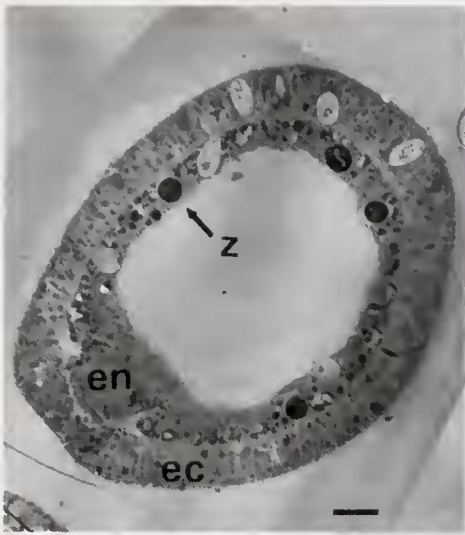
zooxanthellae, all ingested particulate matter was digested or expelled by the following day. When larvae were about 4 days old, they assumed a ball shape, ceased active swimming, and began creeping slowly over the substrate. Starting on day 5, the ball-shaped larvae began to settle. They spread out over the substrate and metamorphosed into volcano-shaped polyps, which began to develop tentacle buds several days after metamorphosis (Fig. 2C).

#### Acquisition of zooxanthellae and onset of symbiosis

Prior to the development of a functional mouth on day 3, planulae of *F. scutaria* did not become infected by experimentally added zooxanthellae. Once the mouth was functional, however, the planulae were able to acquire zooxanthellae. When stimulated to feed, larvae indiscriminately ingested any particulate matter, including experimentally added zooxanthellae. Zooxanthellae either were ingested as part of a larger mass that was fully engulfed by the mouth, or they adhered to mucous strands that were ingested by the larvae. Figure 3A shows a zooxanthella adhered to a larval mucous strand, and Figure 3B shows several zooxanthellae surrounding and contained within the oral cavity of a larva. One hour after zooxanthellae were added, larvae were sampled and fixed for transmission electron microscopy. Figure 4 shows a representative planula 1 h postfeeding, in longitudinal section, with several algae resident in endodermal



**Figure 3.** Scanning electron micrographs detailing zooxanthella acquisition by 3-day-old *Fungia scutaria* planulae. (A) Feeding planula with zooxanthella adhered to mucous strand (m = mouth, z = zooxanthella). (B) Feeding planula, with multiple zooxanthellae entering the mouth. Larvae were fixed for electron microscopy 1 h after exposure to zooxanthella isolates and homogenized *Artemia* (see Methods). Bars = 10  $\mu\text{m}$ .



**Figure 4.** Transmission electron micrograph of a longitudinal section through a *Fungia scutaria* larva infected with zooxanthellae. Thickened oral end at lower left. Zooxanthellae appear in the endoderm as dark spheres. Light ellipses, mostly in the ectoderm, are poorly preserved nematocysts. ec = ectoderm, en = endoderm, z = zooxanthella. Bar = 20  $\mu$ m.

cells. Micrographs suggest that zooxanthellae are phagocytosed by endodermal cells lining the coelenteron (Fig. 5A, B) and appear in both endodermal (Fig. 5C) and ectodermal tissue (Fig. 5D). Although zooxanthellae were still present in ectoderm 24 h later, we did not determine how long zooxanthellae remained within the ectoderm or whether they eventually migrated into the endoderm or were digested or expelled from the host.

Larvae were not limited to forming an association with a specific strain of zooxanthellae; planulae were capable of becoming infected by zooxanthellae isolated from *F. scutaria* (Treatment C2) and *Aiptasia pallida* (Treatment D2), as well as by cultured zooxanthellae from *Cassiopeia xamachana* (Treatment E2) (see Table 1). To determine whether the host had retained zooxanthellae, larvae from Treatments C2 and D2 were observed over a period of 10 to 14 days. Larvae that had acquired zooxanthellae on day 3 remained infected as they progressed through development and metamorphosis into polyps.

Infection by zooxanthellae was not required for metamorphosis: both zooxanthellate and azooxanthellate larvae successfully settled and metamorphosed into polyps (Fig. 6). Larvae infected with zooxanthellae from *F. scutaria* (Treatment C2) and *A. pallida* (Treatment D2) both underwent metamorphosis (we did not monitor settlement for larvae infected with zooxanthellae cultured from *C. xamachana*). Aposymbiotic polyps were able to ingest experimentally added zooxanthellae *via* ciliary currents produced by the polyps that swept particulate matter, including zooxanthellae, over and into their mouths. Observations over the 6

days following showed that the zooxanthellae were retained within the polyps throughout this period.

The proportion of larvae that became infected by zooxanthellae isolated from adult *F. scutaria* (Treatment C) depended on the strength of the feeding response. Feeding was observed to be strongly stimulated (*i.e.*, virtually all larvae began to feed) by the addition of homogenized *Artemia*, but was also stimulated to a lesser extent (*i.e.*, some larvae began to feed) simply by the addition of zooxanthella-isolates, which contained residual animal host tissue. We quantified the effect of larval feeding strength on zooxanthella acquisition for treatments C1 (zooxanthellae alone) and C2 (zooxanthellae and *Artemia*). In the zooxanthellae alone treatment,  $25.0\% \pm 0.02\%$  ( $n = 2$ ) of larvae acquired zooxanthellae, whereas  $96.8\% \pm 0.01\%$  ( $n = 2$ ) became infected when exposed to both zooxanthellae and *Artemia*. It was clear that larvae in Treatments D and E also became infected at a higher rate when exposed to both zooxanthellae and homogenized *Artemia* than to zooxanthellae alone, although the results were not quantified.

An experiment in 1996 provided preliminary evidence that symbiotic state may influence developmental events in *F. scutaria*. Zooxanthellate larvae settled and metamorphosed earlier than azooxanthellate larvae, most of which became arrested in the "ball stage" and then eventually died (Fig. 7). However, the same experiment repeated in 1997 showed low rates of metamorphosis for both zooxanthellate and azooxanthellate larvae and no difference in the timing of metamorphosis.

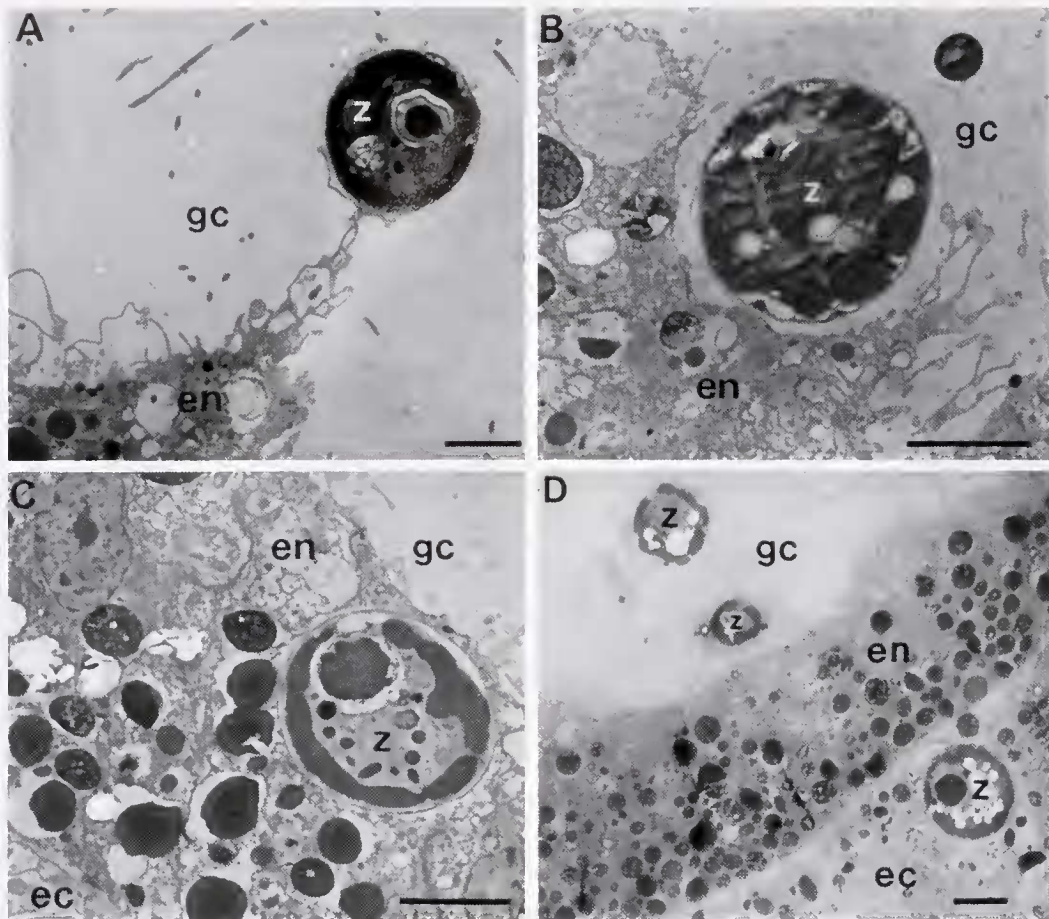
#### *Larval protein profiles*

Protein profiles of larvae through development showed changes with the age of the larvae. Two bands, at 84 and 79 kilodaltons (kDa), were abundant in eggs and 1-day-old larvae (Fig. 8A). As shown in Figure 8B, this protein doublet comprised a significant proportion (36%) of total protein in 1-day-old larvae, but was almost absent by day 6. The apparent depletion of this protein corresponds to the onset of settlement and metamorphosis. The abundance of the putative yolk protein did not differ between 6-day-old azooxanthellate and zooxanthellate larvae.

## Discussion

### *Larval development and acquisition of zooxanthellae*

Development in *Fungia scutaria* was similar to that reported in other broadcast-spawning species of coral (Babcock and Heyward, 1986; review in Harrison and Wallace, 1990). Planula larvae had fully developed within 24 h after fertilization, which is within the range of one to several days reported for other species. Larvae of *F. scutaria* were about 100  $\mu$ m long, ciliated, and barrel-shaped; they exhibited active swimming behavior until they settled at an age of 5



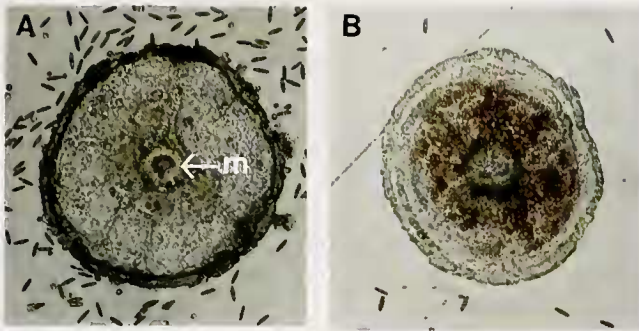
**Figure 5.** Transmission electron micrographs of onset of symbiosis between *Fungia scutaria* planulae and zooxanthellae. (A) Section through endoderm and gastric cavity of a planula showing initial contact between an endodermal cell and a zooxanthella. Host endodermal membranes are very closely associated with the alga. (B) Endodermal cell partially surrounding a zooxanthella, suggesting that the alga is being ingested by the host cell. (C) Zooxanthella resident within a vacuole in an endodermal cell. (D) Two zooxanthellae in gastric cavity (one is being phagocytosed) and one resident within a vacuole in an ectodermal cell. gc = gastric cavity ec = ectoderm, en = endoderm, z = zooxanthella. Bars = 5  $\mu$ m.

days to approximately 2 weeks. This appearance and behavior is typical for externally developed planula larvae.

Very little is known about the feeding ability or behavior of coral planulae. Although it appears that many species, particularly brooding species, produce a nonfeeding larva, the ability to feed has probably gone unrecognized in some species because rearing techniques generally do not expose larvae to a source of particulate food. We found that the feeding behavior of *F. scutaria* was very similar to that reported for the temperate coral *Caryophyllia smithii* (Tranter *et al.*, 1982) and for the temperate sea anemones *Anthopleura elegantissima* and *A. xanthogrammica* (Siebert, 1974; Schwarz, 1996). Feeding consisted of a mouth-opening response to the addition of ground animal tissue, as well as secretion of mucous strands that trapped particulate matter for ingestion.

Although most scleractinian coral species spawn azoo-

xanthellate gametes that develop into azooxanthellate planulae (review in Richmond, 1997), little is known about how planulae might acquire zooxanthellae from the environment. The results of this study support the idea that for corals, competency for infection by zooxanthellae may generally depend on the development of a functional mouth. We found that *F. scutaria* did not become infected by experimentally added zooxanthellae until after a mouth developed. Once the mouth was functional, all developmental stages were competent to become infected. Reports of infection events in other species support this hypothesis—species that are infected at the polyp stage appear to have a nonfeeding planula that does not develop a mouth until the polyp stage (Kinzie, 1974; Babcock and Heyward, 1986; Benayahu *et al.*, 1989). Studies of the feeding behavior of planulae also support this hypothesis: planulae of both *F. scutaria* (this study) and *A. elegantissima* (Schwarz, 1996)

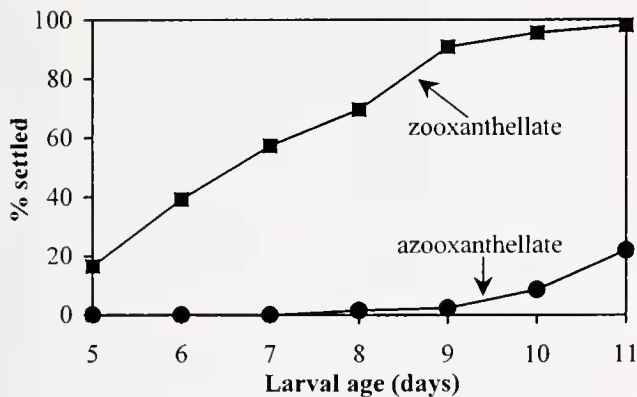


**Figure 6.** Light micrographs of newly settled polyps of *Fungia scutaria*. (A) Azooxanthellate polyp (m = mouth). (B) Zooxanthellate polyp. Zooxanthellae appear as brown spheres in the polyp. The two polyps shown in this figure were settled in the same dish, adjacent to one another. Contaminating diatoms appear as small ellipses around the polyps. Polyp diameter = 100  $\mu$ m.

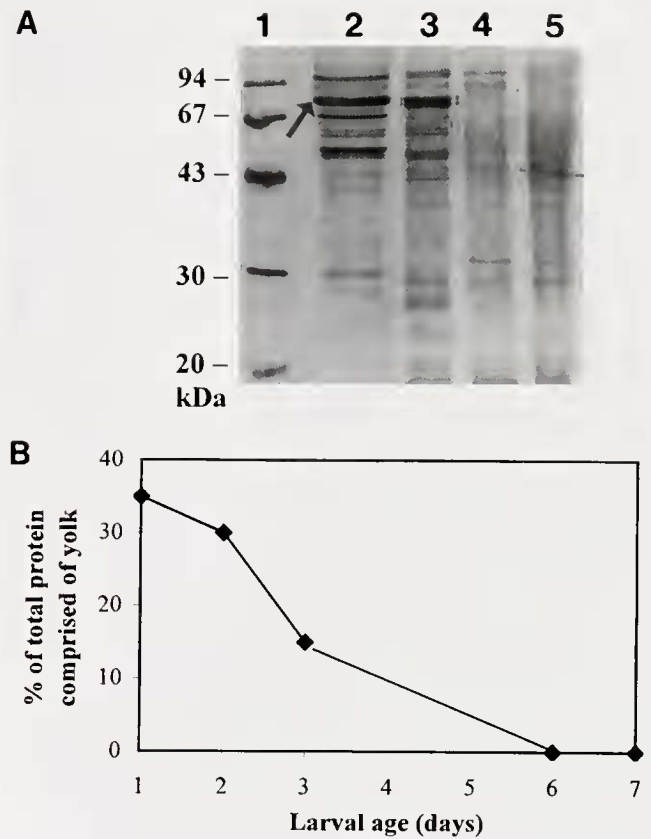
exhibit feeding behavior that leads to the ingestion of zooxanthellae. It will be interesting to determine whether other species that produce a feeding planula larva acquire zooxanthellae in the same manner as shown for *F. scutaria* and *A. elegantissima*.

Both endodermal and ectodermal cells incorporated zooxanthellae within 1 h after larvae were exposed to zooxanthellae. The appearance of zooxanthellae in ectodermal tissue was surprising because zooxanthellae phagocytosed by endodermal cells would not be expected to be transported into tissues where they do not ultimately reside. We did not determine how the zooxanthellae entered the ectoderm. Future work will include long-term sampling of newly infected larvae to investigate the fate of the ectodermal zooxanthellae.

Horizontal transmission of symbionts would appear to be disadvantageous for obligately symbiotic species because of



**Figure 7.** Effect of symbiotic state on larval settlement. Results from 1996 experiment. Nearly 100% of zooxanthellate planulae underwent settlement and metamorphosis by day 10, whereas most azooxanthellate planulae failed to settle. Each point represents data pooled from six replicate dishes for each treatment.



**Figure 8.** Protein profiles and abundance of putative yolk protein in *Fungia scutaria* larvae. (A) Silver-stained 1D SDS-polyacrylamide gel of total protein extracted from eggs (lane 2), 1-day-old larvae (lane 3), 6-day-old azooxanthellate larvae (lane 4), and 6-day-old zooxanthellate larvae (lane 5). Each lane contained 1.2  $\mu$ g protein. Molecular weight standards in lane 1. Arrow highlights a putative yolk protein doublet (84 and 79 kDa) that is abundant in eggs and 1-day larvae but absent by day 6. (B) Decline in abundance of putative yolk protein through larval development. The depletion of putative yolk protein corresponded with the onset of settlement on day 7. There was no difference in putative yolk protein abundance in azooxanthellate and zooxanthellate larvae (days 5 and 6); data shown represent the average of the two treatments.

the possibility that infection may not occur. However, for planulae dispersed to areas with different environmental conditions, the ability to acquire zooxanthellae from the environment might confer a greater advantage to the host than directly inheriting maternal zooxanthellae. This study found that planulae of *F. scutaria* were capable of forming an association with members from three clades of zooxanthellae classified by Rowan and Powers (1991a, b); zooxanthellae from *C. xamachana* are in group A, those from *A. pallida* are in group B, and those from *F. scutaria* are in group C. The degree to which zooxanthellae from different clades persist in *F. scutaria* remains to be investigated, but our results suggest considerable flexibility in host-symbiont specificity in this species. In contrast, planulae of *A. elegantissima*, although able to form an association with zooxan-



thellae recently isolated from a conspecific adult, were unable to do so with cultured *S. californium*, which is the species reported to occur in *A. elegantissima* (Banaszak *et al.*, 1993; Schwarz, 1996).

The finding that a stronger larval feeding response resulted in higher rates of infection indicates that larval feeding behavior may play an important role in acquiring zooxanthellae from the ambient environment. Because so little is known about the distribution and abundance of zooxanthellae in the natural environment, it is difficult to speculate on potential sources of these symbionts. However, one source that is likely to occur in abundance is mucus expelled by corals. Cnidarian hosts regularly expel mucus containing high concentrations of zooxanthellae (Steele, 1975; McCloskey *et al.*, 1996; Schwarz, pers. obs.), and increased rates of expulsion have been reported to accompany spawning (Montgomery and Kremer, 1995; D. Krupp, pers. obs.). Although this study did not examine whether planulae of *F. scutaria* will feed on coral mucus, planulae of the sea anemone *Anthopleura elegantissima* did feed on mucus expelled by adults and became infected by the zooxanthellae within it (Schwarz, 1996). These results suggest that ingestion of zooxanthellae could occur either at the spawning site or at the sites in which the larvae ultimately settle, allowing them to acquire symbionts adapted to different environments.

#### *Effect of symbiont acquisition on larval development*

Zooxanthellae are known to affect the physiology of their adult hosts, and the acquisition of zooxanthellae by larval hosts probably influences larval development. For example, the acquisition of symbionts may act as a settlement cue. An experiment in 1996 demonstrated that zooxanthellate larvae settled earlier than azooxanthellate larvae (Fig. 6)—indeed, most azooxanthellate larvae failed to settle. However, the same experiment repeated the following year showed no differences in settlement (data not shown). It is possible that symbiotic state does influence developmental events but either acts in concert with, or is overridden by, environmental variables such as temperature. The 1996 experiment was conducted during a period of anomalously warm water temperatures that induced a bleaching event on the reef flat in Kaneohe Bay, whereas the 1997 experiment was characterized by normal temperatures. Thus larval development may have been influenced more strongly by water temperature than by symbiotic state. Experimental manipulation of environmental parameters will allow us to examine this question in more detail.

#### *Potential effect of symbiont acquisition on larval energetic strategies and dispersal*

The larval stage serves as a means for dispersal in the life histories of sessile marine invertebrates. The length of the

larval stage depends in part on the amount of energy available for metabolism (Boidron-Métairon, 1995; Levin and Bridges, 1995). Larvae of *F. scutaria* have several potential sources of energy that may allow them to extend the larval stage sufficiently to explain their widespread occurrence throughout the Pacific. First, larvae may initially obtain nutrition from yolk protein supplied through the egg. The presence and the pattern of decline of two abundant 84 kDa and 79 kDa proteins and the correlation between their depletion and the onset of settlement suggest that larvae may metabolize this protein over the course of their development. Second, once the mouth has developed, larvae may obtain energy through feeding. Third, larvae that have acquired zooxanthellae may receive nutrition in the form of organic carbon translocated by zooxanthellae. Richmond (1981, 1987) demonstrated that symbiotic planulae of the coral *Pocillopora damicornis* received about 13%–27% of the carbon fixed by zooxanthellae. Each of these modes of nutrition may operate at different times in development, and each may function to extend the length of the dispersal stage.

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